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Agronomic and Kernel Compositional Traits of Blue Maize Landraces from the Southwestern United States

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RESEARCH

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ABSTRACT

Diverse landraces of maize have been cultivated for centuries in the southwestern United States and northern Mexico primarily for human food consumption. A striking feature of these landraces is the wide array of kernel colors displayed. Traditional cultivation is declining, but blue maize has received increasing commercial interest due to rising consumer demand for unique food products with health benefits and special culinary uses. We evaluated grain yield, agronomic and morphological traits, and analyzed the kernel biochemical composition of five blue and one purple landraces representative of diversity in the Southwest. These were compared with selected open-pollinated populations derived from Southwest and Corn Belt blue maize at several New Mexico locations in 2012 and 2013. Kernel amino acids, oil, protein, starch, fatty acids, crude fiber, ash and anthocyanin pigment contents were determined. Grain yield across all locations, years, and accessions averaged 2.11 Mg ha^{-1}. Navajo Blue and Hopi Blue were the highest and lowest yielding accessions, respectively. The majority of southwestern landraces displayed higher oil content, and two displayed higher protein content, than the Corn Belt Dent variety. Little variation in total amino acid content was observed. Several southwestern floury accessions displayed ~10% greater lysine and methionine than did dent or flint genotypes. Considerable variation for plant, ear, and kernel compositional traits within and across southwestern landraces was consistent with the presence of racial admixtures. The health-promoting properties of anthocyanin-rich landraces contribute to sound dietary nutrition and human health. This study further illustrates the diversity of southwestern maize and supports the rationale for their continued conservation through sustained cultivation and utilization. Directed selection to improve grain yield and uniformity will be necessary to enhance their potential for commercial production.

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Abbreviations: ASI, the anthesis to silking interval; DTS, days to 50% silking; DTA, days to 50% anthesis; EH, ear height; OPV, open-pollinated variety; PH, plant height; QPM, quality protein maize.

Maize (*Zea mays* L.) is a global cereal crop of multiple uses. In the United States, proprietary maize hybrids produce grain used primarily for animal feed and biofuel. This is in contrast with the historic role of maize as a staple food crop for human consumption. In the 1930s and 1940s, open-pollinated varieties (OPVs) became less prevalent in the United States as breeding strategies shifted from improving farmer varieties to the development of suitable inbreds for double-cross, and subsequently single-cross, hybrids (Duvick, 2005). Studies have shown the average superiority of newly introduced hybrids over OPVs to be as much as 15 to 20% (Duvick and Cassman, 1999; Pixley, 2006). In many regions of the world, hybrids have completely supplanted OPVs. Although modern varieties are high yielding, socio-cultural and socio-economic interests still encourage farmers to use landrace, or improved, OPVs. Cultural and personal taste preferences often call for the use of local varieties in the preparation of traditional foods (Pixley and Bänziger, 2004). Lack of availability, or the expense of input resources, also contribute to continued use of landraces and improved OPVs (Bellon et al., 2003; Phiri et al., 2003). In different regions of the world, significant areas of land are still apportioned

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to OPVs (Edgerton, 2009). The continued cultivation of OPVs provides vital in situ conservation of maize genetic diversity (Soleri and Smith, 1995).

Heirloom varieties are landraces of maize passed down by generations of farmers, often in a limited region. They are still grown, primarily on small farms, in the border region between the southwestern United States and northern Mexico (Ryu et al., 2013). In New Mexico, 32% of farms range in size from 1 to 15 ha (USDA, 2012), and traditional OPVs are produced, especially in Hispanic and Native American rural communities, on limited acreage. In this Borderland region, landraces of blue maize are popularly used in various dishes such as atole, posole, pinole, chicos, porridge, tortillas, and tortilla chips (Betrán et al., 2000). Heirloom landraces of maize have retained cultural significance in Native American communities and important meaning may be associated with kernel color for socio-cultural reasons (Wall and Masayesva, 2004; Adams et al., 2006; Werth, 2007). Phenotypic variation for kernel color has been used by farmers to distinguish between, and maintain diversity within, landraces that are preferred for specific traditional uses (Gibson, 2009; Jaradat, 2013). The cultivation of landraces in traditional southwestern farming systems is now practiced primarily by elders, and these traditional systems are in jeopardy of further decline (Nabhan, 2008).

It has been stated that modern interest in blue maize can often be traced to the southwestern United States (Dickerson, 1990; Betrán et al., 2000). Regrettably, the surviving landraces of Borderland maize germplasm have been understudied and underutilized by the plant genetic resources community. In addition to their dietary significance, these landraces may provide a rich source of genetic diversity for utilization in modern breeding programs seeking to develop specialty products (Keleman and Hellin, 2009; Warburton et al., 2008), or for gene discovery using modern techniques including "allele mining" (Bhullar et al., 2009; Jaradat, 2013; Sood et al., 2014). Southwestern maize germplasm may provide alleles for kernel compositional traits of benefit for human health and nutrition (Dickerson, 1991; Kang et al., 2013) and for adaptation to abiotic stresses such as heat, drought, and salinity (Day et al., 1972). A dehydration-responsive element-binding protein (*dehyd1A*) was recently identified as the top population branch statistics outlier in an archeological maize sample from Tularosa Cave, New Mexico dating to 750 yr before present (da Fonseca et al., 2015). This protein has been shown to be upregulated as much as 50-fold in maize roots under drought conditions (Liu et al., 2013). Several New Mexico blue maize varieties displayed elevated concentrations of protein, zinc, iron and flavonoids, particularly anthocyanin, and lysine when compared to commercial yellow dent corn varieties (Dickerson, 1991; Betrán et al., 2000; Ford, 1999).

The objectives of our study were to evaluate the agronomic performance and kernel biochemical composition of a representative sample of blue and purple maize landraces of the US Southwest. Our hypotheses were that grain yields of unselected landraces would be lower than those a selected southwestern OPV ('Los Lunas High') and a Corn Belt Dent OPV ('Ohio Blue'), and that kernel compositional trait values would not differ from those of the selected open-pollinated populations. We also wished to characterize agronomic traits so that their variation and potential for broader utilization could be determined.

MATERIALS AND METHODS

Landrace Accessions

We selected six landrace accessions representative of diverse geographic areas and cultural associations in Arizona and New Mexico. The origin of these accessions is spread across the Colorado Plateau ('Hopi Blue' and 'Navajo Blue'), the southern Rocky Mountains ('Flor del Rio', 'Santa Clara Blue', and 'Taos Blue') and the Southern Range and Basin ('Yoeme Blue'). Taos Blue (NS/S ZM03–015), Yoeme Blue (NS/S ZM01–011), Hopi Blue (NS/S ZM02–147) and Flor del Rio (NS/S ZP-093 Popcorn) were generously provided by Native Seeds/SEARCH (NS/S), Tucson, AZ. Navajo Blue was purchased from Plants of the Southwest, Santa Fe, NM. We compared these accessions with selected OPVs 'Los Lunas High' and 'Ohio Blue'. Los Lunas High was developed through mass-selection at the Agricultural Science Center, Los Lunas, NM (G. Dickerson, New Mexico State Univ., 2011) from the Santa Clara Blue accession. Ohio Blue was obtained from Ohio State University, where it was developed from a cross between 'Ned's Blue' and 'Blue Clarage' at the Ohio Agricultural Research and Development Center, Wooster, OH. Most of these accessions have blue pigmented kernels and floury endosperm with the following exceptions: the Ohio Blue kernel phenotype is dent, and Flor del Rio appears to be an admixture consisting primarily of small flint with some popcorn kernel types. Flor del Rio contains primarily reddish purple kernels, but also a variety of additional kernel colors, i.e., red, red-striped, blue and white (Fig. 1).

Fig. 1. Diversity of Flor del Rio kernels at Alcalde, NM.

Field Evaluations

The OPVs were planted at New Mexico State Univ. Agricultural Science Centers in different regions of New Mexico. In 2012, three locations were selected: Las Cruces (Southern Range and Basin; altitude 1191 m; 32.31° N, −106.77° W; Brazito sandy loam), Los Lunas (Southern Rocky Mountains; altitude 1480 m; 34.80° N, −106.73° W; Vinton loam) and Alcalde (Southern Rocky Mountains; altitude 1741 m; 36.09° N, −106.05° W; Fruitland sandy loam). In 2013, four locations were used for field evaluations: Las Cruces, Los Lunas, Alcalde, and also Farmington (Colorado Plateau; altitude 1615 m; 36.73° N, −108.22° W; Doak sandy loam). The locations are either certified organic (Alcalde and Los Lunas) or were in transition to organic certification (Las Cruces and Farmington). Research plots followed chile (*Capsicum annuum* L.), multiple beans of *Phaseolus spp*., various medicinal herbs, or potato (*Solanum tuberosum* L.) at Las Cruces, Los Lunas, Alcalde, and Farmington, respectively. Plots of 6.1-m length with 1 m between rows were hand-planted with two maize seeds per hill and no thinning was done. Hills were spaced every 0.45 m at all locations, except at Alcalde where hills were spaced 0.6 m apart. At Los Lunas, plots were planted with a mechanical cone-planter to achieve the same plant density (42,748 seeds ha^{-1}). Plants at the Los Lunas and Alcalde locations were fertilized with composted horse and aged cattle manure at rates of 13.8 and 16.1 Mg ha^{-1}, respectively. Alaska Brand fish emulsion fertilizer (Lilly Miller Brands, Walnut Creek, CA) was applied at Las Cruces, a low-input site, at a rate of 74.8 L ha^{-1}. All field plots were irrigated as needed by surface (furrow) irrigation except at Farmington where a center pivot irrigation system was used. Weed control was afforded by mechanical cultivation until canopy cover, after which plots were hand-hoed as needed. All plants within a 3 m subplot (not including end-plants) were hand-harvested. A replicated randomized complete block design was used at all locations. In 2012, two replications were used at Los Lunas and Alcalde and three replications were used at Las Cruces. In 2013, three replications were used at all locations. Yield data were not obtained from Alcalde in 2012 because of bird damage.

Analysis of Pre- and Post-Harvest Phenotypic Traits

Plant and ear height measurements were determined at the Las Cruces location only. Days to 50% silking, anthesis, and the anthesis to silk interval were determined in 2013. Ear traits were evaluated from all 2012 and 2013 locations. Whole ears (with husk leaves attached) were dried using forced ambient air until grain reached 12 to 15% moisture content. Dehusked ears were hand-shelled in 2012; in 2013, they were shelled using a mechanical single-ear sheller (model SCS-2; Agriculex, Guelph, Canada).

Kernel Compositional Analysis

Kernel samples for laboratory analysis were prepared by bulking 100 representative kernels of each accession, from each replicate plot, at each location planted in 2013. Milling was done with a micro hammer cutter mill/Polymix (Glen Mills, Inc., Clifton, NJ) and a 0.5-mm screen. Each sample was milled for approximately one minute (Ryu et al., 2013). All milled samples were stored in amber colored bottles at room temperature. All anthocyanin extracts were stored in a freezer.

Nineteen kernel biochemical compositional traits were analyzed at the Experiment Station Chemical Laboratory, University of Missouri, Columbia, MO from samples produced at all four study locations in 2013. Oil, protein, starch, total fatty acids, crude fiber, ash, anthocyanin content, and twelve amino acids: alanine, cysteine, methionine, lysine, aspartic acid, glutamic acid, glycine, proline, threonine, valine, isoleucine, and leucine were measured. Oil and protein contents were determined using Association of Official Analytical Chemists, International (AOAC) methods 920.39 (A) and 990.03. Starch content was analyzed using approved American Association of Cereal Chemists base method 76-13. Total fatty acids were analyzed by AOAC official methods 996.06 and Ca 5b-71. Crude fiber and ash were analyzed using American Oil Chemists Society (AOCS) approved procedure Ba 6a-05 of AOAC official method 978.10 and AOAC official method 942.05, respectively. Anthocyanins were analyzed according to the method of Li et al. (2011). Amino acids were measured using AOAC method 994.12.

Statistical Analysis

Analyses of variance for grain yield, number of kernel rows per ear, kernels per row, and 100-kernel weight were determined for each location separately as well as for all locations combined. Analysis of variance was performed with PROC GLM in SAS v.9.3 (SAS Institute, 2002). Landrace accessions were considered as fixed effects, and locations, years and the interaction between locations and years were considered as random effects; interactions between accession and location were also tested. Means were compared by least significant difference (LSD) using Fisher's mean separation test.

To provide a fuller examination of the germplasm, multivariate analysis was conducted separately for a group of morphological and developmental traits of agronomic interest, and a group of kernel compositional traits, using principal components analysis (PCA) procedures of MetaboAnalyst 3.0 (Xia et al., 2015). The agronomic traits group included nine pre- and post-harvest traits. The pre-harvest traits were: ear height, plant height, number of ears per plant, anthesis to silking interval (ASI), days to 50% anthesis (DTA) and days to 50% silking (DTS). Post-harvest traits included: number of kernel rows per ear, number of kernels per row and 100-kernel weight. Ten kernel compositional traits examined were: cysteine, methionine, lysine, total fatty acids, protein, oil, crude fiber, ash, starch and anthocyanin content. The data were projected into first and second principal components (PC1 and PC2) to illustrate intra- and inter-landrace variation.

RESULTS

The mean grain yield of all accessions across all locations and years was low (2.11 Mg ha^{-1}) and yields of all southwestern accessions, except Hopi Blue, were comparable to the selected OPVs (Table 1). Yields of Ohio Blue, an OPV assumed to be un-adapted to the Southwest, were equal to those of the locally adapted southwestern accessions. In 2012, the main effect for accessions was statistically significant, and locations by accession interaction effects were

Table 1. Mean values of grain yield of southwestern blue maize grown in Las Cruces, Los Lunas, Farmington, and Alcalde, New Mexico in 2012 and 2013.

Accession	Yield in 2012		Yield in 2013				Yield across locations and years
	Las Cruces	Los Lunas	Las Cruces	Los Lunas	Farmington	Alcalde	
	Mg ha^{-1}						
Navajo Blue	1.55	3.79	1.55	1.54	3.54	2.18	2.36
Santa Clara Blue	1.27	3.18	1.10	1.06	2.65	2.93	2.03
Flor Del Rio	1.23	3.89	1.75	1.87	3.22	2.10	2.34
Yoeme Blue	1.26	1.65	2.11	2.43	2.77	2.09	2.05
Hopi Blue	1.52	2.09	1.98	1.35	2.35	1.50	1.63
Taos Blue	1.39	1.79	1.87	1.78	2.17	2.24	1.87
Los Lunas High	1.00	1.84	1.49	2.03	3.95	3.22	2.26
Ohio Blue	2.12	2.15	1.32	2.81	3.42	2.11	2.32
Average	1.42	2.55	1.65	1.86	3.01	2.30	2.11
Range	1.00–2.12	1.65–3.89	1.10–2.11	1.06–2.81	2.17–3.95	1.50–3.22	1.63–2.36
LSD	0.64	2.14	1.02	1.47	1.61	0.77	0.50

Table 2. Mean squares of grain yield of southwestern blue maize grown in Las Cruces, Los Lunas, Farmington, and Alcalde, New Mexico in 2012 and 2013, across years and locations.

	Across years and locations		2012		2013	
	df	Grain yield	df	Grain yield	df	Grain yield
Replication	2	0.68	2	0.61	2	1.63*
Accession (A)	7	1.59*	7	1.93**	7	1.23*
Location (L)	3	14.16***	1	14.48***	3	9.07***
A × L	21	0.73	7	2.01***	21	0.61
Year (Y)	1	17.10***				
A × Y	7	1.85**				
Y × L	1	7.25***				
A × L × Y	7	1.65**				
Error	80	0.51	22	0.36	56	0.54

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 3. Mean values of phenotypic traits† of southwestern blue maize across four locations in New Mexico in 2012 and 2013.

Accession	DTA‡	DTS‡	ASI‡	PH§	EH§	Ears per plant§	Kernel rows¶	Kernels per row¶	100-kernel weight¶
	d			cm		No.			g
Navajo Blue	52.0	62.0	10.0	166.9	78.3	2.3	12.2	35.8	31.8
Santa Clara Blue	57.3	66.0	8.7	178.3	82.4	1.9	15.0	33.9	21.7
Flor Del Rio	53.3	64.7	11.3	165.6	90.7	2.6	14.2	30.3	22.7
Yoeme Blue	54.7	63.3	8.7	106.6	34.3	1.7	14.8	31.1	26.1
Hopi Blue	54.7	62.0	7.3	111.9	37.8	1.6	12.5	30.0	27.4
Taos Blue	52.0	62.0	10.0	161.1	67.2	2.0	14.9	36.8	22.6
Los Lunas High	54.7	66.0	11.3	171.0	86.4	1.6	15.2	35.4	25.3
Ohio Blue	54.7	64.7	10.0	173.2	82.3	2.0	15.5	32.8	29.1
Average	54.2	63.8	9.7	154.3	69.9	2.0	14.3	33.3	25.8
Range	52.0- 57.3	62.0–66.0	7.3–11.3	106.6–178.3	34.3–90.7	1.6–2.6	12.2–15.5	30.0–36.8	21.7–31.8
LSD	2.80	4.36	3.88	15.43	13.97	0.66	0.92	3.49	2.25

† Abbreviations of plant traits: DTA, days to 50% anthesis; DTS, days to 50% silking; ASI, anthesis to silking interval; PH, plant height; EH, ear height.

‡ Data collected from Las Cruces in 2013.

§ Data collected from Las Cruces in 2012 and 2013.

¶ Data collected from Las Cruces, Los Lunas, Alcalde, and Farmington in 2012 and 2013.

also significant. In 2013, no significant interactions were seen between accessions and locations (Table 2). Significant interaction between accessions by year and accessions by year by location suggests that the grain yield performance of these landraces varied depending on the specific test environment. As anticipated, the lowest grain yields were observed at the Las Cruces location, which is managed as a low fertility site.

More variation for DTA was observed than for DTS. An average of 54.2 DTA and 63.8 DTS was reported with an average 9.7 d of ASI for all entries (Table 3). All accessions were prolific to some extent (range 1.6 to 2.6 ears plant^{-1}) and most accessions displayed ear height at or slightly below mid-plant height (average primary ear height of 69.9 cm and 154.3 cm plant height across all accessions). Considerable variation for plant height was

Table 4. Mean squares of yield components of southwestern blue maize grown at Las Cruces, Los Lunas, Farmington, and Alcalde, New Mexico in 2012 and 2013.

	Across years and locations				2012				2013			
	df	Kernel rows	Kernels per row	100-kernel weight	df	Kernel rows	Kernels per row	100-kernel weight	df	Kernel rows	Kernels per row	100-kernel weight
Replication	2	3.16	40.49	9.61	2	4.76	61.88	6.92	2	3.46	18.40	4.44
Accession (A)	7	20.2***	139.9***	156.2***	7	17.8***	148.76***	156.7***	7	16.54***	90.9***	84.3***
Location (L)	3	12.2***	447.5***	274.6***	2	4.77	73.39	266.6**	3	9.70***	481***	132.8***
A × L	21	4.60***	56.87***	15.94	14	5.9	88.13*	39.04*	21	2.0	27.81	11.55
Year (Y)	1	0.58	4.25	81.27**								
A × Y	7	3.35	107.05***	13.22								
L × Y	2	3.16	121.9***	169.3***								
A × L × Y	14	4.48*	53.5**	22.61*								
Error	78	1.70	22.17	10.75	30	3.34	28.97	15.70	62	1.29	18.31	10.73

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 5. Mean squares of plant, ear, and flowering traits of southwestern blue maize grown in Las Cruces, New Mexico across years, in 2012 and 2013.

		df	Ears per plant	Ear height	Plant height	Days to 50% anthesis	Days to 50% silking	Anthesis to silking interval
Across years	Accession (A)	7	1.1**	2901.0***	751.1**			
	Replication	2	0.40	132.4	62.02			
	Year (Y)	1	8.33***	1025.8**	5.33			
	A × Y	7	1.0*	290.4**	750.3**			
	Error	30	0.26	90.4	152.5			
2012	Accession	7	1.80**	2251.5***	4495.1***			
	Replication	2	0.04	57.04	319.7			
	Error	14	0.23	130.4	252.4			
2013	Accession	7	1.80	2251.5***	4495.08***	23.78***	39.78***	13.50*
	Replication	2	0.04	0.44	319.7	6.0	11.16	1.50
	Error	14	0.23	130.4	252.4	2.57	6.21	4.93

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

observed. During both years of study, Hopi Blue and Yoeme Blue had the lowest, and Santa Clara Blue the tallest, average plant height, with a difference of over 70 cm.

All post-harvest ear traits: number of kernel rows per ear; number of kernels per row and 100-kernel weight were evaluated at three locations in 2012 and at four locations in 2013. Across all locations and years, the average 100-kernel weight was 25.8 g; ears averaged 33.3 kernels row^{-1} and 14.3 rows ear^{-1} (Table 3). Navajo Blue displayed the highest and Santa Clara the lowest 100-kernel weight. The maximum kernels per row were observed in Taos Blue and the lowest in Flor del Rio. Ohio Blue and Navajo Blue had the highest and lowest number of kernel rows, respectively. The average number of kernel rows was similar across locations whereas the number of kernels per row and the 100-kernel weight were variable across locations. During 2012, only number of kernels and 100-kernel weight showed significant interaction between accession and location whereas during 2013, accessions and locations were significant for all yield components, but no interaction was seen between accession and location (Table 4). In years 2012 and 2013, the number of ears, ear height, plant height, DTA, DTS, and ASI were statistically different among accessions. Across both years, the number of ears, ear height, and plant height were significantly different among accessions and also showed significant interaction between accession and year (Table 5).

When averaged across all environments, oil was 6.3% (on a dry wt. basis), protein was 12.3%, starch was 61.0%, total fatty acid was 6.1%, crude fiber was 1.8%, ash was 1.7%, and anthocyanins were 452 mg kg^{-1} (Table 6). Most notable was the high oil content of all southwestern accessions, except Flor del Rio. Oil and total fatty acid contents of Santa Clara Blue and Hopi Blue were highest, and of Flor del Rio and Ohio Blue, were lowest. The highest oil and protein contents were observed in Los Lunas and least amounts at Farmington and Alcalde, respectively. Starch content was higher in Las Cruces and least in Farmington. Crude fiber was fairly similar at all locations and total fatty acids and anthocyanins were highest in Los Lunas and least in Farmington. Santa Clara Blue was highest for crude fiber, ash and anthocyanins whereas Hopi Blue displayed the lowest level of crude fiber and Flor del Rio displayed the lowest levels of ash and anthocyanins. The accessions displayed significant differences for all kernel biochemical components, except starch content (Table 7).

Table 6. Mean values of kernel biochemical traits of southwestern blue maize evaluated during 2013. Data were collected from Las Cruces, Los Lunas, Alcalde, and Farmington, New Mexico.

Accession	Amino acids	Oil	Protein	Starch	Fatty acids	Crude fiber	Ash	Anthocyanin
	% dry wt.							mg kg^{-1}
Navajo Blue	9.23	6.41	12.1	61.4	6.19	1.71	1.63	471
Santa Clara Blue	9.79	7.30	12.9	60.5	7.04	1.94	1.82	595
Flor Del Rio	9.33	3.96	12.2	60.0	3.82	1.73	1.57	74
Yoeme Blue	9.20	6.85	12.2	61.4	6.61	1.79	1.71	565
Hopi Blue	9.44	7.45	12.3	60.8	7.18	1.67	1.70	558
Taos Blue	10.02	6.89	13.2	61.8	6.66	1.70	1.73	362
Los Lunas High	9.03	6.92	11.8	59.7	6.68	1.88	1.67	479
Ohio Blue	9.08	4.74	11.9	61.9	4.57	1.76	1.50	513
Average	9.39	6.32	12.3	60.9	6.09	1.77	1.67	452
Range	9.03–10.02	3.96–7.45	11.8–13.2	59.7–61.9	3.82–7.18	1.67–1.94	1.50–1.82	74–595
LSD	0.61	1.17	0.73	2.31	1.09	0.11	0.08	78.1

Table 7. Mean squares of kernel biochemical traits of southwestern blue maize grown at four locations in New Mexico during 2013.

		df	Across locations	Las Cruces	Los Lunas	Farmington	Alcalde
Total amino acids	Accession (A)	7	1.43**	0.60	0.61*	1.07	0.94
	Replication	2	0.46	0.32	0.46	0.71	0.39
	Location (L)	3	2.89**				
	A × L	21	0.57				
	Error	59	0.54	0.31	0.21	0.49	1.17
Oil	Accession (A)	7	18.73***	6.15**	12.50**	5.94*	4.28*
	Replication	2	0.76	0.80	4.59	1.17	1.39
	Location (L)	3	4.87				
	A × L	21	3.15*				
	Error	59	0.78	1.23	2.56	1.99	1.31
Protein	Accession (A)	7	2.54*	1.06	0.70	1.38	1.52
	Replication	2	0.24	0.51	0.41	0.23	0.80
	Location (L)	3	5.14***				
	A × L	21	0.68				
	Error	59	1.84	0.43	0.52	0.50	1.67
Starch	Accession (A)	7	8.42	3.94	15.53	1.02	0.49
	Replication	2	2.74	1.09	10.6	8.04	11.21
	Location (L)	3	32.68*				
	A × L	21	6.73				
	Error	59	7.69	5.41	10.65		5.23
Total fatty acids	Accession (A)	7	17.41***	5.72**	11.67**	5.55*	3.97*
	Replication	2	0.69	0.72	4.37	1.09	1.33
	Location (L)	3	4.55				
	A × L	21	2.95*				
	Error	59	1.72	1.14	2.38	1.86	1.23
Crude fiber	Accession (A)	7	0.10***	0.09***	0.03	0.02	0.03
	Replication	2	0.05	0.02	0.01	0.02	0.02
	Location (L)	3	0.02				
	A × L	21	0.02				
	Error	59	0.02	0.01	0.02	0.01	0.04
Ash	Accession (A)	7	0.11***	0.03**	0.04**	0.04**	0.03
	Replication	2	0.005	0.003	0.01	0.002	0.01
	Location (L)	3	0.06**				
	A × L	21	0.01				
	Error	59	0.01	0.01	0.01	0.01	0.02
Anthocyanin	Accession (A)	7	3353.3***	834.2***	1523.5***	672.8***	1292.2***
	Replication	2	100.2	9.43	137.9	150.1	3.06
	Location (L)	3	1115.5***				
	A × L	21	302.7***				
	Error	59	88.15	92.50	109.5	70.78	85.52

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 8. Mean values and ratios between essential amino acids (cysteine, methionine, and lysine) of southwestern blue maize evaluated in 2013. Data were collected from Las Cruces, Los Lunas, Alcalde, and Farmington, New Mexico.

Accession	Cysteine	Methionine	Lysine	Cysteine/protein ratio	Methionine/protein ratio	Lysine/protein ratio
	g kg^{-1}			%		
Navajo Blue	2.46	2.99	3.53	2.05	2.49	2.90
Santa Clara Blue	2.52	3.40	3.71	1.96	2.64	2.87
Flor Del Rio	2.43	2.78	3.43	2.01	2.29	2.81
Yoeme Blue	2.39	3.03	3.67	1.98	2.50	3.02
Hopi Blue	2.43	3.12	3.63	1.89	2.52	2.95
Taos Blue	2.48	2.93	3.82	1.98	2.22	2.90
Los Lunas High	2.33	3.10	3.63	1.97	2.62	3.09
Ohio Blue	2.45	2.83	3.38	2.06	2.35	2.83
Average	2.44	3.02	3.60	1.99	2.45	2.92
Range	2.33–2.52	2.78–3.40	3.38–3.82	1.89–2.06	2.22–2.61	2.81–3.07
LSD	0.12	0.21	0.16	0.09	0.14	0.15

Locations were statistically different for protein, starch, ash and anthocyanins. Across all environments, significant interactions between accession and location were reported for all traits except protein and starch (Table 7).

Essential amino acids methionine and lysine, and the conditionally essential amino acid cysteine, were the least abundant amino acids (data not presented for other amino acids) and only methionine showed much variation across accessions (Table 8). The relationship between different biochemical compositions was examined by comparing the ratios between individual amino acids cysteine, methionine with lysine, with protein (Table 8). These ratios also did not show much variability among different accessions.

Variability generated by the different categorical descriptors of the populations (agronomic and kernel compositional trait groups) was explained by a total of eight principle components following PCA. The PCA biplot for agronomic and kernel compositional traits can be seen in Figures 2 and 3, respectively. Approximately 67 and 93% of the total variation was contributed by principal components 1 (PC1) and 2 (PC2) for agronomic and kernel compositional traits, respectively. The variation contributed by 100-kernel weight, ears per pant, plant height, ear height and ASI were the primary contributors to PC1 variability, whereas flowering traits (DTA and DTS) and kernel rows per ear contributed to the variability of PC2. No distinct groups of landraces were seen for the agronomic traits category. Kernel compositional traits total fatty acids, crude fiber, essential amino acids (cysteine, lysine and methionine), oil, protein and starch all contributed to the variability of PC1, and only anthocyanin contributed variability to PC2. In comparison to agronomic traits, kernel compositional traits displayed more variability, although no clearly distinct groups were observed.

DISCUSSION

We conducted research with blue maize accessions in different geographical areas of NM that are representative of different growing regions in the southwestern United States. Observed yields were consistent with those reported in other studies using similar management practices (Adams et al., 2006; Werth, 2007). The selected OPVs Los Lunas High and Ohio Blue offered no demonstrative advantages over the majority of the southwestern landraces. Higher yields appeared to be associated with high-altitude (cooler) sites; however, lower yields at the low altitude site (Las Cruces), perhaps attributable to heat stress, might also have been associated with low soil fertility. Muenchrath et al. (2002) studied open-pollinated "folk cultivars" at four different locations on the Zuni Indian Reservation (Pueblo of Zuni) in NM and reported a mean grain yield of 0.57 Mg ha^{-1} in a traditional rainwater cultivation system. Our management practices represented a compromise between traditional systems characterized by low inputs and plant population densities, and high input-high density production systems. Kutka (2011) reported that OPVs could yield >2 Mg ha^{-1} and various studies suggested that under optimal growing conditions high yielding OPVs can yield >4.4 Mg ha^{-1} (Belsito, 2004; Kutka et al., 2004). Not surprisingly, our field experiments resulted in yields that were higher than those in the Muenchrath et al. (2002) study, but lower than those reported by Belsito (2004) and Kutka (2011). The results of our study suggest that diverse types of blue maize can be grown in the Southwest, but selection for improved yield and greater uniformity would enhance opportunities for commercial development. Consideration might also be given to development of a hybrid blue maize breeding program to achieve increased yield and suitability for mechanical harvest.

Plant height varied considerably among the accessions. Our results showed Santa Clara Blue as the tallest population with an average height of 178 cm and Hopi Blue as the shortest at 112 cm. Adams et al. (2006) and Soleri and Smith (1995) reported plant height in the range 118 to 137 cm for different accessions of Hopi Blue maize. Hopi Blue from our study was similar to the shortest accessions in their findings. All southwestern accessions displayed prolificacy. Primary ear height tended to be slightly below

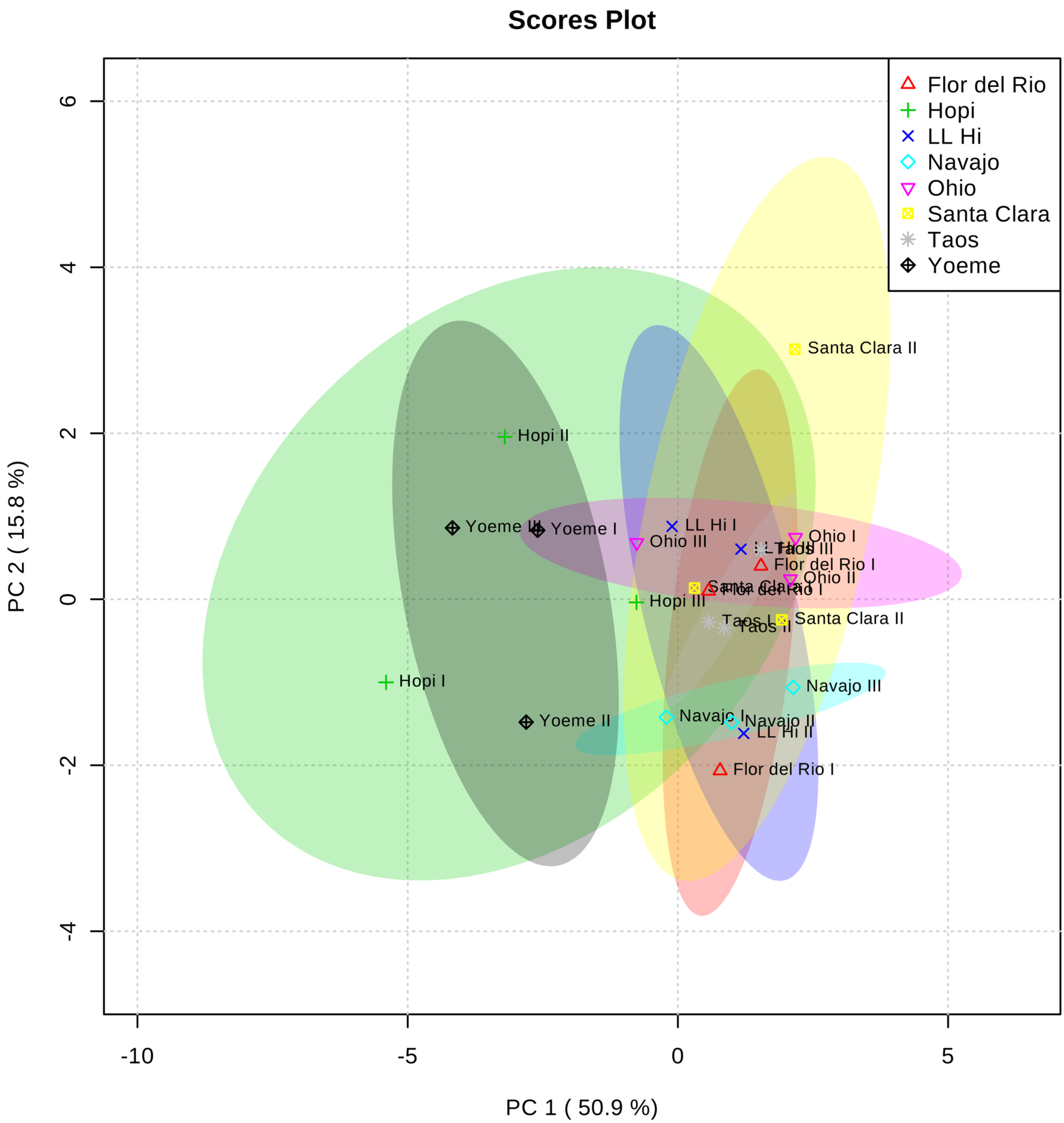

Fig. 2. Principal component analysis (PCA) of agronomic traits. The PCA biplot is composed of principal components 1 and 2. Agronomic trait PCA was evaluated for nine pre- and post-harvest traits.

mid-plant height. These findings were consistent with the results of Adams et al. (2006).

Across all locations, the grand mean for 100-kernel weight in 2012 and 2013 was reported at 25.9 and 25.0 g, respectively. In our study, the average range for 2012 and 2013 was 21.1 to 33.1 g and 21.4 to 30 g, respectively, demonstrating the anticipated variation associated with variable kernel types. Werth (2007) reported the 100-kernel weights for Navajo Blue, Yoeme Blue, and Flor del Rio to be 26.4, 23.9, and 20.4 g, respectively, and our results have shown similar, but proportionally higher, 100-kernel weights of 31.8, 26.1, and 22.7 g, respectively. Comprehensive studies of diverse southwestern landraces showed similar results for kernels per row (Werth, 2007 and Adams et al., 2006). Variation for kernel size, row number, and kernels/ear might be useful in the selection of male and female inbred seed parents if hybrid development is pursued as a breeding objective.

Morphological traits used in our study have also been used to examine races of southwestern maize. Our

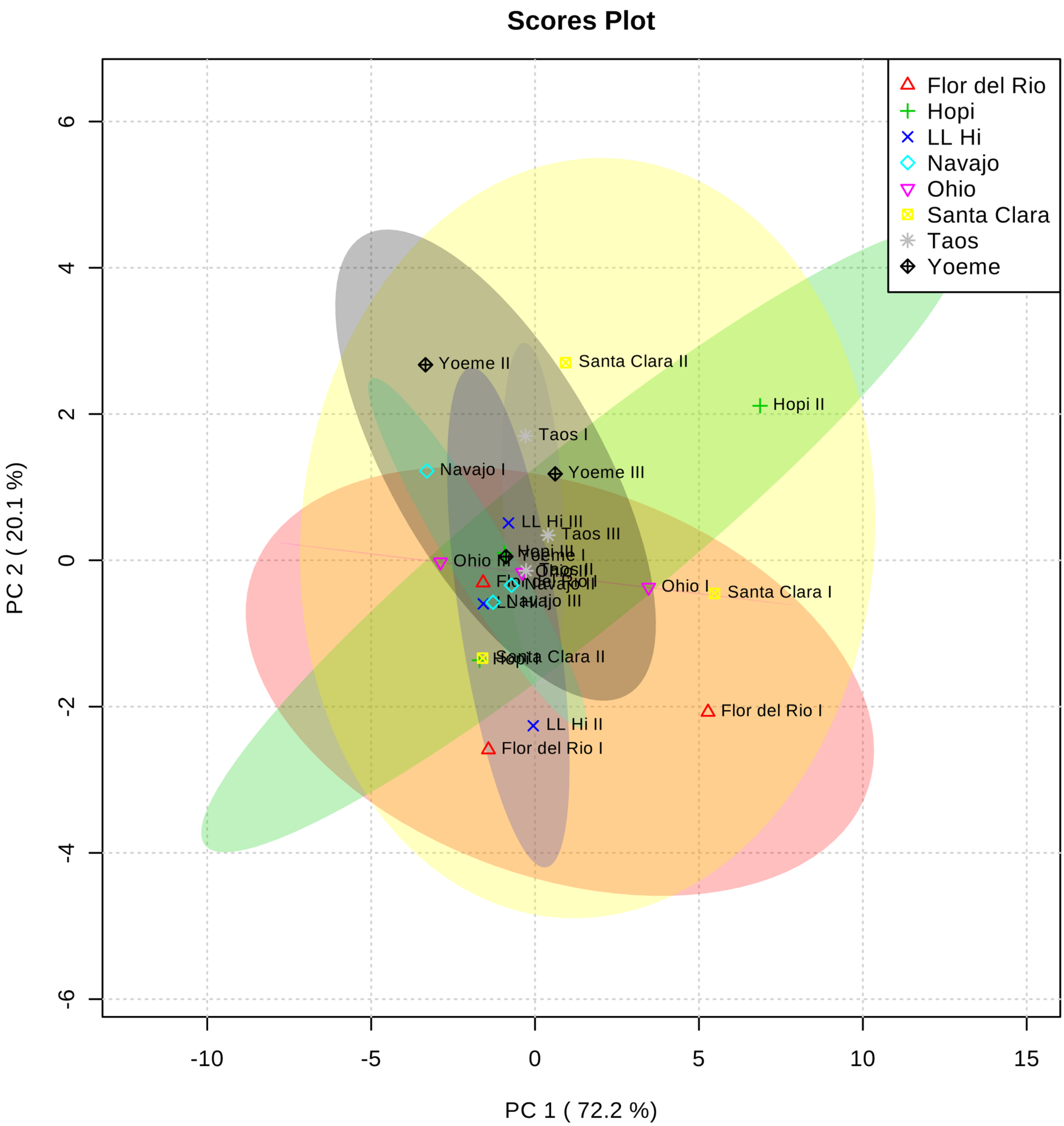

Fig. 3. Principal component analysis (PCA) of kernel compositional traits. PCA biplot is composed of principal components 1 and 2. Kernel compositional trait PCA was evaluated for ten diverse traits.

observations of these traits are consistent with the classifications assigned by Native Seeds/SEARCH to the landraces provided for this study, with the exception of Flor del Rio, which appears to be an admixture of multiple kernel types and not popcorn per se (Werth, 2007; Ryu et al., 2013). Yoeme Blue is representative of Pima-Papago race and Hopi Blue belongs to the 12-row Southwestern/Hopi race (Werth, 2007; Ryu et al., 2013). Navajo Blue, Santa Clara Blue, Los Lunas High and Taos Blue have not been classified into any specific racial group although their phenotypes are generally consistent with the descriptors ascribed to Puebloan maize (Carter and Anderson, 1945; Doebley et al., 1983). Those authors considered Puebloan maize to likely be an admixture of different races. The presence of multiple races may facilitate the identification of heterotic groups suitable for development of hybrid maize varieties.

Most of the kernel compositional trait values were significantly higher than those of the selected OPVs. Two landraces, Santa Clara Blue and Taos Blue, were each identified as higher for several compositional traits. The protein and oil contents of the southwestern maize accessions, except Flor del Rio, were higher than those of Ohio Blue. Flint-Garcia et al. (2009) showed that many maize landraces were higher in protein and oil than modern inbreds. Protein and oil values of landraces thus would appear to be higher than those reported for Corn Belt hybrids in multiple studies (Watson, 2003; Belyea et al., 2004; Dudley et al., 2007; Jaradat and Goldstein, 2013) and quality protein maize hybrids (Zarkadas et al., 2000). The crude fiber and ash content were reported to be lower in blue maize compared to yellow dent maize (Wu et al., 2006). In comparison with blue maize, commercial hybrids and modern varieties were reported to be higher in starch (Ridley et al., 2002; Scott and Blanco, 2009), which is consistent with the intense selection for higher grain yield in commercial hybrids. Kernel color is dependent on anthocyanin amount and several studies have shown that red and purple pigmented kernels are lower in anthocyanin content than blue kernels (Lopez-Martinez et al., 2009; Zhao et al., 2009; Ryu et al., 2013; Collison et al., 2015). The lower anthocyanin content of red and purple vs. blue Flor del Rio kernels is consistent with those studies.

Galinat (1977) considered (floury) varieties native to the Southwest and arid regions of Latin America to contain the *fl1* gene. Zarkadas (1997) reported that a Great Lakes regional landrace variety of floury maize displayed amino acid profiles similar to those of an *fl2* genotype. It displayed slightly elevated lysine values, and twofold higher methionine levels, when compared to a northern flint inbred. The lysine content in *fl1* mutants and wild-type maize is usually about 2%, and reaches approximately 4% of total protein, in *fl2* and *o2* mutants (Weizmann, 1998). Azevedo et al. (2003) also showed *fl2 and o2* mutants to be higher in lysine content (3.8% of total protein) than *fl1,* and *fl1* to be most similar to, wild-type maize (1.9 vs. 1.5% of total protein, respectively). Levels of lysine from our study were somewhat higher than those of *fl1* genotypes reported in the above studies; however, the majority of floury OPV lysine values were only about 10% higher those of the dent or flint OPVs we examined. Lysine levels of floury Peruvian maize landraces were also shown to have lysine levels that did not differ from ordinary corns (Alexander and Elmore, 1968). Methionine levels of the OPVs tested in our study were similar to the values reported by Phillips et al. (2008) for high-methionine Corn Belt Dent maize inbreds (2.25 to 3.45 g kg^{-1}). Methionine levels in floury maize examined by Zarkadas (1997) were higher than those of *fl2* genotype and twofold higher than those observed in wild-type flint maize. Three floury accessions in our study showed methionine levels that were approximately 10% higher than those of the dent and flint OPVs. These values appear most similar to those reported for the *fl1* genotype. Further research will be necessary to confirm if the genetic basis for the floury phenotype of southwestern blue maize endosperm is similar to that of known floury mutations.

Doebley et al. (1983) conducted principal coordinate analysis of 45 accessions of maize landraces from the Southwest using isozyme analysis of 12 enzyme systems encoded by 22 loci. The authors concluded that no distinct clusters existed among the accessions due to significant overlap among the diverse landraces chosen to reflect all major linguistic and cultural groups of Native Americans in the Southwest. We also observed a high degree of overlap between all landraces and the selected OPVs following PCA for both agronomic and kernel compositional traits. These results are consistent with the prior descriptions of heterogeneity for morphological traits and supposition that the majority of landraces representing the Native American Pueblos in northern Arizona and New Mexico are interracial admixtures (Anderson and Cutler, 1942; Doebley et al., 1983). We also note that Hopi Blue appeared to be the most variable landrace for agronomic traits. Expression of agronomic traits in Ohio Blue and Navajo Blue was less variable. Ohio Blue is a selected variety so this is not surprising. The reduced variation exhibited by Navajo Blue may also be associated with the fact that it is commercially available, and may have been subjected to selection for uniformity. Santa Clara Blue showed considerable variation for kernel compositional traits.

CONCLUSIONS

A representative sample of accessions of southwestern blue maize OPVs displayed considerable variation for agronomic traits and modest variation for kernel compositional traits. Multivariate analysis of agronomic and kernel compositional traits showed considerable heterogeneity within most southwestern landraces, and considerable overlap among the accessions. Yields of the majority of the accessions did not differ from those of two selected OPVs. The majority of southwestern landraces displayed elevated oil content in comparison to Corn Belt Dent open-pollinated blue maize. Anthocyanin levels in blue maize were higher than those in reddish purple maize. Nutritional and health benefits may accrue from retention, or reincorporation, of these pigmented landraces into the diet (Notah Begay III Foundation, 2015). Suitability for traditional dishes, combined with the opportunity to save seed from OPVs, also provides additional incentives to encourage their continued cultivation. This research establishes a benchmark for further characterization and utilization of diverse southwestern blue maize landraces. Directed breeding efforts will be required to raise grain yields to levels needed for commercial production.

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References

Adams, K.R., C.M. Meegan, S.G. Ortman, R.E. Howell, L. Werth, D.A. Muenchrath, M.K. O'Neil, and C.A.C. Gardner. 2006. MAIS (Maize of American Indigenous Societies) Southwest: Ear descriptions and traits that distinguish 27 morphologically distinct groups of 123 historic USDA maize accessions and data relevant to archeological subsistence models. Arizona State Univ., Tempe, AZ. http://farmingtonsc.nmsu.edu/documents/maissouthwestcopyrightedmanuscript1.pdf (accessed on 1 Nov. 2014; verified 18 Apr. 2016).

Alexander, D.E., and C. Elmore. 1968. Lysine content of Peruvian floury varieties. Maize Gen. Coop. Newsl. 42:41–42.

Anderson, E., and H.C. Cutler. 1942. Races of *Zea mays*. I. Their recognition and classification. Ann. Mo. Bot. Gard. 29:69–88.

Azevedo, R.A., C. Damerval, J. Landry, P.J. Lea, C.M. Bellato, L.W. Meinhardt, G.M. Le, S. Delhaye, A.A. Toro, S.A. Gaziola, and B.D. Berdejo. 2003. Regulation of maize lysine metabolism and endosperm protein synthesis by opaque and floury mutations. Eur. J. Biochem. 270:4898–4908. doi:10.1111/j.1432-1033.2003.03890.x

Bellon, M.R., M. Adato, J. Becerril, and D. Mindek. 2003. The impact of improved maize germplasm on poverty alleviation: The case of Tuxpeno-derived material in Mexico. International Food Policy Research Institute, Washington, DC.

Belsito, A.M. 2004. Open pollinated corn variety trials and a discussion of the practical implications for open pollinated corn in small-scale whiskey production. M.S. Thesis, Cornell Univ., Ithaca, NY.

Belyea, R.L., K.D. Rausch, and M.E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresour. Technol. 94:293–298. doi:10.1016/j.biortech.2004.01.001

Betrán, F.J., A.J. Bockholt, and L.W. Rooney. 2000. Blue corn. In: A.R. Hallauer, editor, Specialty corns. CRC Press, Boca Raton, FL. p. 293–337.

Bhullar, N.K., K. Street, M. Mackay, N. Yahiaoui, and B. Keller. 2009. Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the *Pm3* resistance locus. Proc. Natl. Acad. Sci. USA 106:9519–9524. doi:10.1073/pnas.0904152106

Carter, G.F., and E. Anderson. 1945. A preliminary survey of maize in the southwestern United States. Ann. Mo. Bot. Gard. 32:297–323. doi:10.2307/2394378

Collison, A., L. Yang, L. Dykes, S. Murray, and J.M. Awika. 2015. Influence of genetic background on anthocyanin and copigment composition and behavior during thermoalkaline processing of maize. J. Agric. Food Chem. 63:5528–5538.

da Fonseca, R.R., B.D. Smith, N. Wales, E. Cappellini, P. Skoglund, M. Fumagalli, J.A. Samaniego, C. Caroe, M.C. Avila-Arcos, D.E. Hufnagel, T.S. Korneliussen, F.G. Vieira, M. Jakobsson, B. Arriaza, E. Willerslev, R. Nielsen, M.B. Hufford, A. Albrechts, J. Ross-Ibarra, and M.T.P. Gilbert. 2015. The origin and evolution of maize in the southwestern United States. Nat. Plants 1:14003. doi:10.1038/nplants.2014.3

Day, A.D., D.R. Grove, and R.K. Thompson. 1972. Arizona Indian corn (*Zea mays* L.). J. Ariz. Acad. Sci. 7:8–11. doi:10.2307/40024358

Dickerson, G.W. 1990. Blue corn production and marketing in New Mexico, Guide H-226. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State Univ., Las Cruces, NM.

Dickerson, G.W. 1991. Nutritional analysis of blue corn and dent corn kernels, Guide H-233. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State Univ., Las Cruces, NM.

Doebley, J.F., M.M. Goodman, and C.W. Stuber. 1983. Isozyme variation in maize from the southwestern United States: Taxonomic and anthropological implications. Maydica 28:97–120.

Dudley, J.W., D. Clark, T.R. Rocheford, and J.R. LeDeaux. 2007. Genetic analysis of corn kernel chemical composition in the random mated 7 generation of the cross of generations 70 of IHP × ILP. Crop Sci. 47:45–57.

Duvick, D.N., and K.G. Cassman. 1999. Post-green revolution trends in yield potential of temperate maize in the North-Central United States. Crop Sci. 39:1622–1630. doi:10.2135/cropsci1999.3961622x

Duvick, D.N. 2005. Genetic progress in yield of United States maize. Maydica 50:193–202.

Edgerton, M.D. 2009. Increasing crop productivity to meet global needs for feed, food, and fuel. Plant Phys. 149:7–13. doi:10.1104/pp.108.130195

Flint-Garcia, S.A., A.L. Bodnar, and M.P. Scott. 2009. Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. Theor. Appl. Gen. 119:1129–1142. doi:10.1007/s00122-009-1115-1

Ford, D. 1999. Blue corn unique to American southwest. New Mexico State Univ., All about discovery. News Center, 2 Nov. 1999. New Mexico State Univ. Co-op Ext. Serv. College of Agriculture and Home Economics, Las Cruces, NM. http://newscenter.nmsu.edu/Articles/view/5717 (accessed 20 Jan. 2015).

Galinat, W.C. 1977. The floury-1 and floury-2 genes for improving sweet maize. Maize Gen. Coop. Newsl. 51:43–43. Maize Genetics and Genomics Database, Univ. of Missouri, Columbia, MO.

Gibson, R.W. 2009. A review of perceptual distinctiveness in landraces including an analysis of how its roles have been overlooked in plant breeding for low-input systems. Econ. Bot. 63:242–255. doi:10.1007/s12231-009-9086-3

Jaradat, A.A. 2013. Perceptual distinctiveness in Native American maize (*Zea mays* L.) landraces has practical implications. Plant Genet. Resour. 11(03):266–278. doi:10.1017/S1479262113000063

Jaradat, A.A., and W. Goldstein. 2013. Diversity of maize kernels from a breeding program for protein quality: I. physical, biochemical, nutrient, and color traits. Crop Sci. 53:956–976. doi:10.2135/cropsci2012.07.0437

Kang, M.-K., S.S. Lim, J.-Y. Lee, K.M. Yeo, and Y.-H. Kang. 2013. Anthocyanin-rich purple corn extract inhibit diabetes-associated glomerular angiogenesis. PLoS ONE 8(11):e79823. doi:10.1371/journal.pone.0079823

Keleman, A., and J. Hellin. 2009. Specialty varieties in Mexico: A case study in market-driven agro-biodiversity conservation. J. Latin Am. Geog. 8:147–174. doi:10.1353/lag.0.0061

Kutka, F. 2011. Open-pollinated *vs.* hybrid maize cultivars. Sustainability 3:1531–1554. doi:10.3390/su3091531

Kutka, F., P. Conway, and J. Christensen. 2004. The heritage maize project (FNC00-301) study of open-pollinated corn: Final report to the North Central region Sustainable Agriculture Research and Education Program. USDA SARE Program, College Park, MD. http://mysare.sare.org/sare_project/fnc00-301/ (verified 18 Apr. 2016).

Li, J., C.E. Walker, and J.M. Faubion. 2011. Acidulant and oven type affect total anthocyanin content of blue corn cookies. J. Sci. Food Agric. 91:38–42. doi:10.1002/jsfa.4173

Liu, S., X. Wang, H. Wang, H. Xin, X. Yang, J. Yan and J. Li., L.P. Tran, K. Shinozaki, K.Y. Shinozaki, and F. Qin. 2013. Genome-wide analysis of *ZmDREb* genes and their association with natural variation in drought tolerance at seedling stage of *Zea mays* L. PLoS Gent. 9:e1003790.

Lopez-Martinez, L.X., R.M. Oliart-Ros, G. Valerio-Alfaro, C.-H. Lee, K.L. Parkin, and H.S. Garcia. 2009. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. Food Sci. Tech. 42:1187–1192.

Muenchrath, D.A., M. Kuratomi, J.A. Sandor, and J.A. Homburg. 2002. Observational study of maize production systems of Zuni farmers in semi-arid New Mexico. J. Ethnobiol. 22:1–33.

Nabhan, G.P. 2008. Rediscovering America and surviving the dust bowl: The US southwest. In: G.P. Nabhan, editor, Where our food comes from: Retracing Nikolay Vavilov's quest to end famine. Island Press, Washington, DC. p. 125–138.

Notah Begay III Foundation. 2015. Social determinants of health of type 2 diabetes and obesity in Native American children: A research framework for Notah Begay III Foundations Native Strong: Healthy Kids, Healthy Futures Program. Notah Begay III Foundation, Santa Ana Pueblo, NM.

Phillips, R.L., J. Suresh, M. Olsen, and T. Krone. 2008. Registration of high-methionine versions of maize inbreds A632, B73, and Mo17. J. Plant Reg. 2:243–245.

Phiri, M.A.R., M. Mekuria, and M. Bänziger. 2003. Assessment of smallholder farmers' utilization of improved maize seed in the SADC region: A study of Malawi, Tanzania, Zambia and Zimbabwe. Southern African drought and low fertility project (SADLF). CIMMYT, Harare, Zimbabwe.

Pixley, K.V. 2006. Hybrid and open-pollinated varieties in modern agriculture. In: K.R. Lamkey and M. Lee, editors, Plant breeding: The Arnel R. Hallauer International Symposium. Blackwell Publishing, Ames, IA. doi:10.1002/9780470752708.ch17

Pixley, K., and M. Bänziger. 2004. Open-pollinated maize varieties: A backward step or valuable option for farmers? In: D.K. Friesen and A.F.E. Palmer, editors, Integrated approaches to higher maize productivity in the new millennium: Proc. Seventh Eastern and Southern Africa Regional Maize Conference. 5–11 Feb. 2002. Nairobi, Kenya. CIMMYT and Kenya Agricultural Research Institute, Nairobi, Kenya. p. 22–28.

Ridley, W.P., R.S. Sidhu, P.D. Pyla, M.A. Nemeth, M.L. Breeze, and J.D. Astwood. 2002. Comparison of the nutritional profile of glyphosate-tolerant corn event NK603 with that of conventional corn (*Zea mays L.*). J. Agric. Food Chem. 50:7235–7243. doi:10.1021/jf0205662

Ryu, S.H., L. Werth, S. Nelson, J.C. Scheerens, and R.C. Pratt. 2013. Variation of kernel anthocyanin and carotenoid pigment content in USA/Mexico Borderland landraces of maize. Econ. Bot. 67:98–109. doi:10.1007/s12231-013-9232-9

SAS Institute. 2002. SAS Version 9.2. SAS Inst., Inc., Cary, NC.

Scott, M.P., and M. Blanco. 2009. Evaluation of the grain methionine, lysine, and tryptophan contents of maize (*Zea mays* L.) germplasm in the germplasm enhancement of maize project. Plant Genet. Resour. 7:237–243.

Soleri, D., and S. Smith. 1995. Morphological and phenological comparisons of two Hopi maize varieties conserved in situ and ex situ. Econ. Bot. 49:56–77. doi:10.1007/BF02862278

Sood, S., S. Flint-Garcia, M.C. Wilcox, and J.B. Holland. 2014. Mining natural variation for maize improvement: Selection on phenotypes and genes. In: R. Tuberosa, A. Graner, and E. Frison, editors, Genomics of plant genetic resources. Springer, Dordrecht. Germany. p. 615–649.

USDA. 2012. Census of agriculture, Table 64: Summary by size of farm. http://www.agcensus.usda.gov/Publications/2012/Full_Report/Volume_1,_Chapter_1_State_Level/New_Mexico/st35_1_064_064.pdf (accessed 14 Jan. 2015).

Wall, D., and V.I. Masayesva. 2004. People of the corn: Teachings in Hopi traditional agriculture, spirituality, and sustainability. Am. Indian Q. 28:435–453. doi:10.1353/aiq.2004.0109

Warburton, M.L., J.C. Reif, M. Frisch, M. Bohn, C. Bedoya, X.C. Xia, J. Crossa, J. Franco, D. Hoisington, K. Pixley, S. Taba, and A.E. Melchinger. 2008. Genetic diversity in CIMMYT nontemperate maize germplasm: Landraces, open pollinated varieties, and inbred lines. Crop Sci. 48:617–624. doi:10.2135/cropsci2007.02.0103

Watson, S.A. 2003. Description, development, structure, and composition of the corn kernel. In: P.J. White and L.A. Johnson, editors, Corn: Chemistry and technology. 2nd ed. Am. Assoc. Cereal Chemists, Saint Paul, MN. p. 69–106.

Weizmann, G.G. 1998. Enhancing the content of the essential amino acids lysine and threonine in plants. In: B.K. Singh, editor, Plant amino acids: Biochemistry and biotechnology. Marcel Dekker, Inc, New York. p. 487–508.

Werth, L.C. 2007. Characterization and classification of Native American maize landraces from the Southwestern United States. M.S. Thesis. Iowa State University, Ames, IA.

Wu, X., R. Zhao, D. Wang, S.R. Bean, P.A. Seib, M.R. Tuinstra, M. Campbell, and A. O'Brien. 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. Cereal Chem. 83:569–575.

Xia, J., I. Sinelnikov, B. Han, and D.S. Wishart. 2015. MetaboAnalyst 3.0: Making metabolomics more meaningful. Nucleic Acids Res. 43:w251–w257. doi:10.1093/nar/gkv380

Zarkadas, C.G. 1997. Assessment of the protein quality of native white floury maize, designated IAPO-13, by amino acid analysis. J. Agric. Food Chem. 45:1062–1069. doi:10.1021/jf960573o

Zarkadas, C.G., R.I. Hamilton, Z.R. Yu, V.K. Choi, S. Khanizadeh, N.G.W. Rose, and P.L. Pattison. 2000. Assessment of the protein quality of 15 new northern adapted cultivars of quality protein maize using amino acid analysis. J. Agric. Food Chem. 48:5351–5361. doi:10.1021/jf000374b

Zhao, X., C. Zhang, C. Guigas, Y. Ma, M. Corrales, B. Tauscher, and H. Xiaosong. 2009. Composition, antimicrobial activity, and antiproliferative capacity of anthocyanin extracts of purple maize (*Zea mays* L.) from China. Eur. Food Res. Technol. 228:759–765. doi:10.1007/s00217-008-0987-7